Xenopus oocytes can synthesise but do not secrete the Z variant of human α_1-antitrypsin

R.C. Foreman, J.D. Judah and A. Colman*

Department of Experimental Pathology, University College London, University Street, London WC1E 6JJ and **Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, England*

Received 14 January 1984

Human liver mRNA was prepared from a patient homozygous for α_1-antitrypsin deficiency (PiZZ) and from a normal subject (PiMM). Both liver RNAs were microinjected into *Xenopus* oocytes and α_1-antitrypsin identified by immunoprecipitation. The normal M variant of α_1-antitrypsin is synthesised and secreted by *Xenopus* oocytes, the abnormal Z protein is not secreted and an intracellular form accumulates in the oocytes. In the presence of tunicamycin an unglycosylated form of M α_1-antitrypsin appears in the incubation medium but no corresponding unglycosylated version of the Z protein is secreted.

α_1-Antitrypsin *Human liver mRNA* *PiM variant* Xenopus *oocyte* *PiZ variant* *α_1-Antitrypsin secretion*

1. INTRODUCTION

α_1-Antitrypsin is one of the most important proteinase inhibitors found in human plasma [1]. The glycoprotein is synthesised in the liver, but acts principally in the lung to prevent proteolytic damage to the pulmonary alveoli [1,2]. In view of this protective function it is not surprising that chronic pulmonary disease often arises in patients with inborn deficiency of α_1-antitrypsin [3]. What is surprising is that homozygotes for the genetic variant of α_1-antitrypsin, known as PiZ, frequently sustain damage to the liver as well as the lung [4]. Indeed, the endoplasmic reticulum of affected livers contains large deposits of α_1-antitrypsin [5,6] and this has led to the suggestion that α_1-antitrypsin deficiency of the PiZZ type arises from a failure in secretion. The accumulations of intracellular inhibitor which result may give rise to a susceptibility to further liver damage [1]. DNA sequence studies [7] have shown that a single point mutation of the normal PiM gene gives rise to the PiZ genotype. The mutation causes a glutamic acid to lysine substitution at position 342 in the polypeptide chain but how this alteration prevents the protein from being secreted is unknown. Messenger RNA for PiZ α_1-antitrypsin translates as efficiently as the normal message in a cell-free translation system [8]. Furthermore, the mutant protein is correctly sequestered within dog pancreas microsomes and core glycosylated [9], suggesting that the block in secretion occurs at a later stage in the secretory pathway.

We have chosen to study this problem using *Xenopus* oocytes as a model secretory system. *Xenopus* oocytes are capable of translating a wide variety of exogenous mRNAs [10]. Foreign secretory proteins are modified post-translationally and exported from the oocyte [11,12] which makes it a suitable surrogate system for our purposes.

2. MATERIALS AND METHODS

Liver samples were obtained by biopsy (normals) or as a result of liver transplantation (PiZZ homozygote). Liver samples were cut into small pieces and rapidly frozen and stored in liquid

Published by Elsevier Science Publishers B V

nitrogen. Normal tissue was obtained from several donors and all were phenotyped as PiM. The PiZZ donor was a 7-year-old girl undergoing liver transplantation. Microscopic examination of the liver from the deficient patient revealed intracellular inclusions of α_1-antitrypsin. Oligo(dT) cellulose was from BRL Laboratories (Science Park, Cambridge); L-[^{35}S]methionine (>800 Ci · mmol^{-1}) and ^{14}C-labelled marker proteins were from Amersham International (Amersham, Bucks.); antihuman α_1-antitrypsin raised in rabbits was from DAKO (Copenhagen). All other reagents were analytical grade and were supplied by Sigma (Poole, Dorset).

Liver poly(A$^+$) RNA was prepared by a modification of the method in [13]. Frozen liver samples (2 g) were pulverised in a percussion mortar before homogenisation using a Potter Teflon and glass homogeniser. This homogenisation procedure is particularly important when dealing with PiZZ liver, which tends to be very fibrous. Poly(A$^+$) RNA was separated using oligo(dT) cellulose affinity chromatography. Two g of liver yielded 50–80 μg poly(A$^+$) RNA. Preparation and use of the cell-free translation system were as in [14]. Oocytes of *Xenopus laevis* were maintained in Barths' saline and microinjected as in [12,15]. Oocytes were microinjected with mRNA with or without tunicamycin at 40 μg · ml^{-1}. Injected oocytes were cultured in unlabelled Barth's saline for 24 h at 21°C (~2 μg · ml^{-1} tunicamycin). Unhealthy oocytes were discarded and the remaining oocytes were cultured in Barth's saline containing 0.8 mCi · ml^{-1} [^{35}S]methionine for 7 h. In the pulse chase experiment oocytes were removed from the labelled medium and incubated for a further 24 h in medium containing 10 mM methionine. Oocytes were homogenised according to [15]. Oocyte extracts and incubation media were immunoprecipitated with anti-α_1-antitrypsin as in [16]. Polyacrylamide gels (10%, w/v) and subsequent fluorography were as in [14]. Two-dimensional gel electrophoresis was according to [17].

3. RESULTS AND DISCUSSION

We prepared poly(A$^+$) RNA from the livers of normal and PiZZ patients. Batches of oocytes were then microinjected with either PiMM or PiZZ mRNA and incubated in the presence of medium containing [^{35}S]methionine. Fig.1 (tracks 1 and 2) shows a control immunoprecipitation of non-injected oocytes using anti-α_1-antitrypsin. The bands seen represent non-specific binding to the antibody–protein A–Sepharose complex and were not removed by 'clearing' immunoprecipitations or extensive washing of the complex. When oocytes injected with liver RNA were similarly treated additional bands, specific for α_1-antitrypsin, were clearly visible (tracks 3–6). A 58-kDa

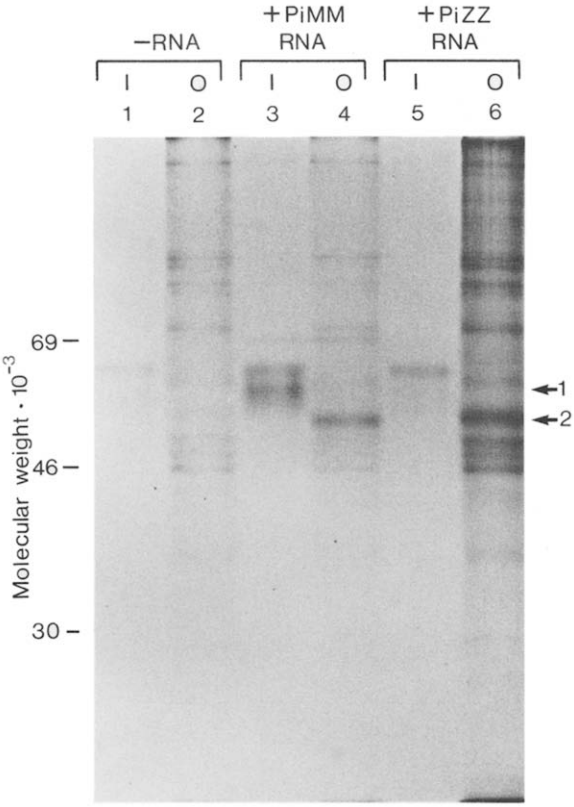

Fig.1. The synthesis and secretion of α_1-antitrypsin in oocytes. Oocytes were microinjected with liver poly(A$^+$) RNA and incubated in medium containing [^{35}S]methionine as described in section 2. Antibody precipitates from incubation media and oocyte extracts were examined by SDS-polyacrylamide gel electrophoresis. I, incubation medium; O, oocyte extract. Arrows 1 and 2 denote the 58-kDa and 54-kDa forms of α_1-antitrypsin referred to in the text. M_r values shown on the left of the figure were derived from the comigration of ^{14}C-labelled markers, carbonic anhydrase (30000), ovalbumin (46000) and bovine serum albumin (69000).

protein was found in the incubation medium from oocytes injected with PiMM mRNA (track 3). This secreted form was usually absent if oocytes were injected with PiZZ mRNA (track 5) although in some experiments a small amount of PiZ α_1-antitrypsin is secreted into the medium (see fig.3, track 7). A 54-kDa protein appeared in oocytes injected with normal or mutant mRNA (tracks 4 and 6). Evidently, little PiZ α_1-antitrypsin is secreted by oocytes but the intracellular precursor appears in all oocytes injected with liver mRNA. The difference between intracellular precursors of PiM and PiZ inhibitor is clearly resolved in the pulse chase experiment shown in fig.2.

Fig.2 displays the result of an experiment when oocytes were labelled for 7 h then 'chased' by transfer to media containing 10 mM methionine

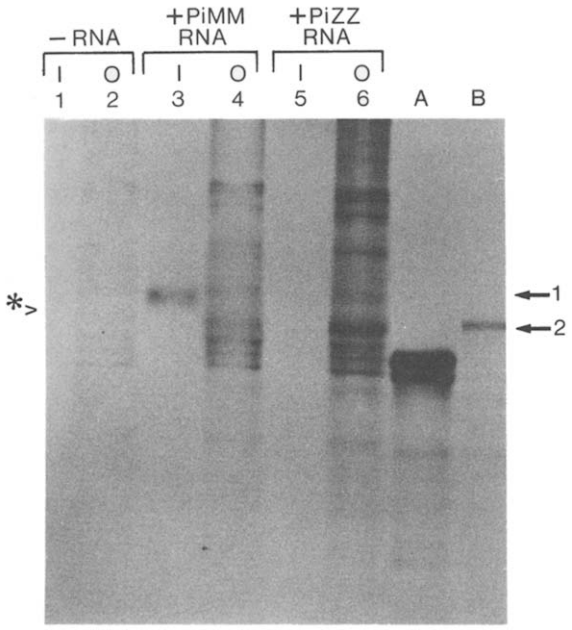

Fig.2. Disposition of α_1-antitrypsin from pulse-labelled oocytes. Oocytes were microinjected with liver poly(A^+) RNA and pulse-labelled as described in section 2. Antibody precipitates were examined by SDS-polyacrylamide gel electrophoresis as before. I, incubation medium; O, oocyte extract. Arrows 1 and 2 denote 58-kDa and 54-kDa forms of α_1-antitrypsin as before. Tracks A and B show α_1-antitrypsin synthesised in a cell-free system after addition of PiMM RNA, with (B) or without (A) membranes. * Migration of unlabelled marker α_1-antitrypsin from human serum.

for a further 24 h. The intracellular PiM α_1-antitrypsin disappeared with concurrent appearance of the secreted protein in the medium (tracks 3 and 4). The PiZ precursor remained in the oocyte during the chase period and little export occurred (tracks 5 and 6) over this period. There appeared to be no intracellular degradation of unsecreted PiZ protein over the chase period. The intracellular form of α_1-antitrypsin migrated slightly faster than the membrane-processed, cell-free translation product (track 8). This may indicate different degrees of core glycosylation in oocytes and canine microsomes or some further sugar modification to the oocyte protein. The total export of PiM α_1-antitrypsin seen in fig.2 is not always observed. In other experiments we have seen only a partial clearing of intracellular precursor over the chase period. These quantitative differences are probably due to batch to batch variation in the oocytes. On no occasion was PiZ α_1-antitrypsin efficiently secreted into the medium. These results suggest that the treatment of normal and abnormal variants of α_1-antitrypsin in the oocyte accurately reflects events in the hepatocytes of normal and PiZZ individuals.

The contribution of attached oligosaccharide units toward glycoprotein secretion is somewhat variable (review [18]). α_1-Antitrypsin is a complex glycoprotein with 3 asparagine-linked oligosaccharide chains per molecule and in the case of the PiZ variant the possibility arises that the attached core sugars may play some part in the block in secretion from the liver. We have investigated the effect of glycosylation on the fate of normal and mutant α_1-antitrypsin by incubating injected oocytes with tunicamycin, an inhibitor of core glycosylation.

In the presence of tunicamycin a new 45-kDa protein is secreted from oocytes microinjected with normal mRNA (fig.3, track 5). This represents secretion of unglycosylated PiM α_1-antitrypsin after cleavage of the signal peptide and is analogous to findings from experiments using rat hepatocytes incubated with tunicamycin [19,20]. Although the unglycosylated protein was found intracellularly in oocytes injected with either normal or mutant mRNA (tracks 6 and 8), there was no corresponding export of an unglycosylated form of PiZ α_1-antitrypsin (track 7). The α_1-antitrypsin which accumulates in the liver of patients

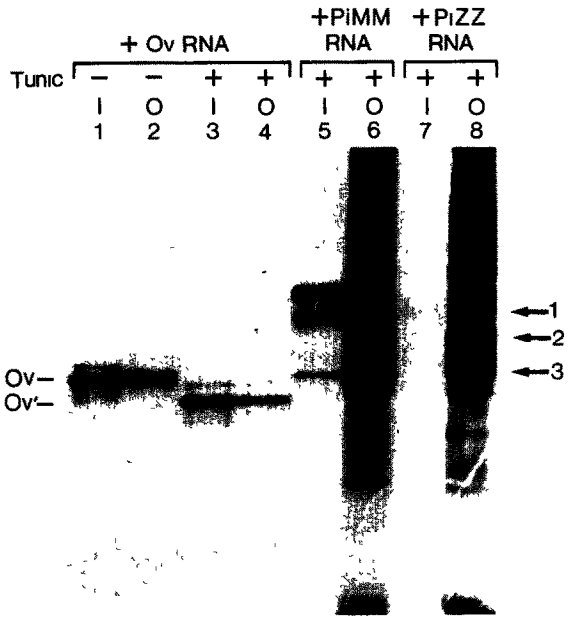

Fig.3. The effect of tunicamycin on protein secretion in oocytes. Oocytes were microinjected with mRNA from human liver or hen oviduct and cultured in the presence or absence of tunicamycin. Antibody precipitates of incubation media and oocyte extracts were prepared using either anti-ovalbumin or anti-α_1-antitrypsin and examined as before. I, incubation medium; O, oocyte extract. Arrows 1,2 and 3 denote 58-kDa, 54-kDa and 45-kDa forms of α_1-antitrypsin referred to in the text. Ov and Ov', glycosylated and unglycosylated forms of ovalbumin.

homozygous for the Z variant is at least partly core glycosylated and has a high mannose content [21]. It is possible that decreased solubility of the intracellular abnormal protein is the cause of the failure to secrete. However, we can say that the addition of core sugars plays little or no part in this decreased solubility since tunicamycin did not alleviate the block in export from oocytes.

The tunicamycin treatment was not completely effective as judged by the presence of the 54-kDa intracellular band in tracks 6 and 8. However it is interesting to note that, in contrast to the intracellular situation, in the media the glycosylated and not the unglycosylated form of α_1-antitrypsin predominates (track 5). We conclude therefore that the fully glycosylated molecule is exported from the oocyte far more rapidly than the unglycosylated molecule. This effect of glycosylation on secretion rate did not extend to another protein, chick ovalbumin. As shown in [11] the secretion rate of ovalbumin is not affected by glycosylation (cf. fig.3, tracks 1 and 2,3 and 4). At present we cannot explain the intense band of about 66-kDa seen in fig.3, track 5. This band was seen in other experiments but its intensity relative to α_1-antitrypsin was much lower. This band was only seen in media surrounding oocytes injected with PiMM RNA.

Our results show that the failure to process correctly and secrete PiZ α_1-antitrypsin is not confined to the hepatocyte. Also, we show that the abnormal glycosylation in itself is not the cause of the secretory block since unglycosylated PiZ protein is not secreted. One might wonder why an aberrant protein is not correctly translocated once it has entered the secretory pathway. A similar situation has been reported in studies on a mutant $\lambda 2$ light chain [22]. A one-residue substitution blocks secretion of the globulin and it accumulates in the myeloma cell. This $\lambda 2$ light chain is also not secreted in monomer form from *Xenopus* oocytes after mRNA microinjection [23]. In the case of α_1-antitrypsin a simple answer may be that the mutant inhibitor has a reduced solubility and aggregates in the endoplasmic reticulum. However, there remain two other interesting possibilities. One, that the mutation in the protein causes its diversion to some cellular compartment of the liver not on the secretory pathway and the second that the aberrant protein fails to bind to a receptor in the endoplasmic reticulum which is required for correct transport. These suggestions are open to experimental investigation.

ACKNOWLEDGEMENTS

This work was supported by grants to R.C.F. and J.D.J. from the Wellcome Trust and the Vandervell Foundation and to A.C. from the Cancer Research Campaign. We gratefully acknowledge the invaluable assistance of Dr Benito Lombardi and Dr David Van Thiel of the School of Medicine, University of Pittsburgh. We also thank Professor C. Clark, Department of Surgery, University College London.

REFERENCES

[1] Carrell, R.W. and Owen, M.C. (1979) Essays Med. Biochem. 4, 83–119
[2] Lieberman, J. (1976) Chest 70, 62–67.
[3] Laurell, C.B. and Eriksson, S. (1963) Scand. J. Clin. Lab. Invest. 15, 132–140.
[4] Sharp, H.L , Bridges, R.A., Krivit, W. and Freier, E.F. (1969) J. Lab. Clin. Med. 73, 934–939.
[5] Sharp, H.L. (1971) Hosp. Pract. 6, 83–96.
[6] Yunis, E.J., Agostini, R.M. jr and Glew, R.H. (1976) Am. J. Pathol. 82, 265–286.
[7] Kidd, V.J., Wallace, R.B., Itakura, K and Woo, S.L.C. (1983) Nature 304, 230–234
[8] Errington, D.M., Bathurst, I.C., Janus, E.D and Carrell, R.W. (1982) FEBS Lett. 148, 83–86.
[9] Bathurst, I.C., Stenflo, J., Errington, D.M. and Carrell, R.W. (1983) FEBS Lett. 153, 270–274.
[10] Lane, C D. (1983) Curr Top Dev Biol. 18, 89–116.
[11] Colman, A., Lane, C.D., Craig, R., Boulton, A., Mohun, T. and Morser, J. (1981) Eur. J. Biochem. 113, 339–348
[12] Colman, A. and Morser, J. (1979) Cell 17, 517–526.
[13] Chirgwin, J.M., Przybyla, A.E., MacDonald, R J. and Rutter, W.J. (1979) Biochemistry 18, 5294–5299.
[14] Foreman, R.C. and Judah, J.D. (1982) Biosci. Rep. 2, 995–1002.
[15] Colman, A. (1984) in: Transcription and Translation, a Practical Approach (Hames, B.D. and Higgins, S. eds) IRL Press, in press
[16] Dobberstein, B., Garoff, H. and Warren, G. (1979) Cell 17, 759–769.
[17] O'Farrell, P.H. (1975) J. Biol. Chem. 250, 4007–4021.
[18] Olden, K., Parent, J.B. and White, S.L. (1982) Biochim. Biophys. Acta 650, 209–232.
[19] Gross, V., Geiger, T., Tran-Thi, T.-A., Gauthier, F. and Heinrich, P.C. (1982) Eur. J. Biochem. 129, 317–323.
[20] Carlson, J. and Stenflo, J. (1982) J Biol. Chem 257, 12987–12994.
[21] Hercz, A., Katona, E., Cutz, E , Wilson, J R. and Barton, M. (1978) Science 201, 1229–1232.
[22] Wu, G.E., Hozumi, N. and Murialdo, H. (1983) Cell 33, 77–83.
[23] Valle, G., Beasley, J., Williamson, A., Mosmann, T. and Colman, A. (1983) Eur. J. Biochem 132, 131–138